Carbon costs and compromises

Neven, Ika

CHAPTER 3

Dissolved CO_2 manipulation experiments with natural phytoplankton communities from the Southern Ocean:

I. Effects on growth parameters

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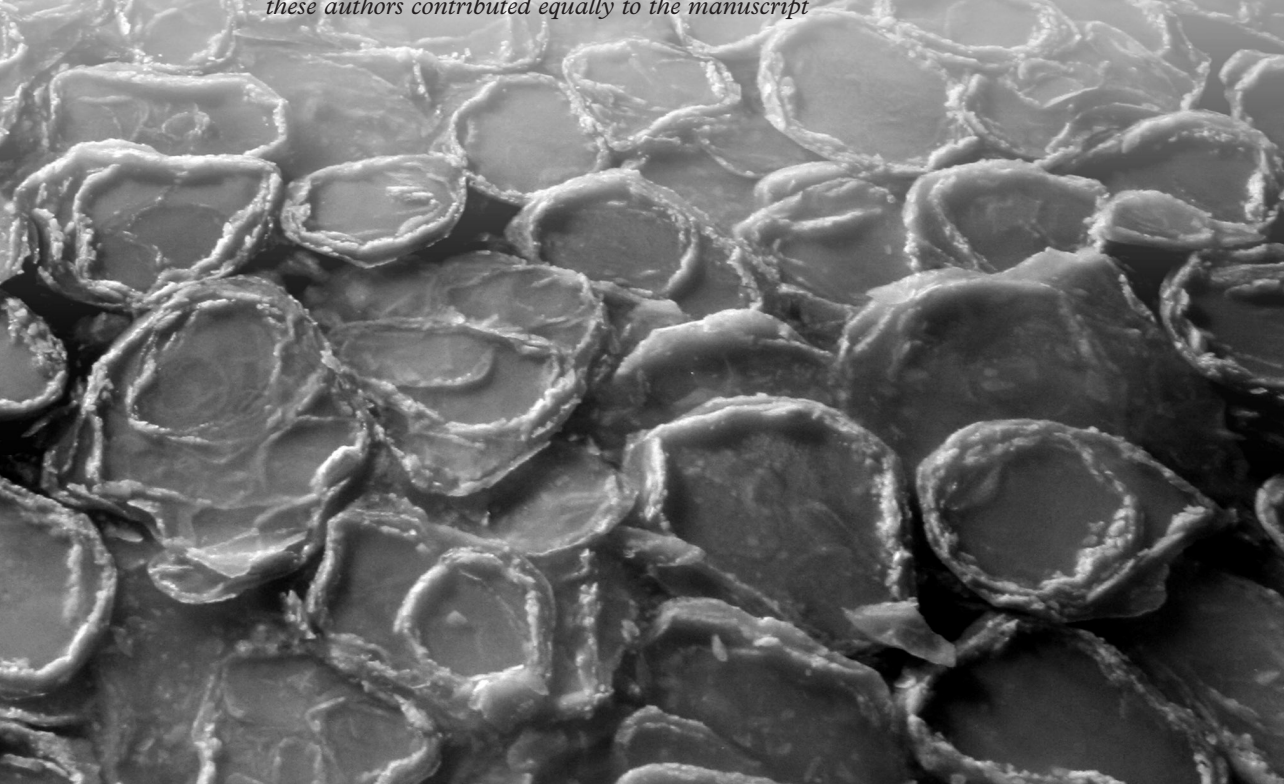


ABSTRACT

Experiments with natural phytoplankton communities were performed under low (~190 µatm), present (~380 µatm) and high (~750 µatm) pCO_2 conditions during a field campaign to the Atlantic sector of the Southern Ocean in February - April 2008. Growth, photosynthetic efficiency, pigment composition, particulate organic carbon (POC) accumulation, nutrients and carbon uptake were investigated during 6 incubation experiments with water from the Polar Front, the Antarctic Circumpolar Current, the Weddell Sea and the Drake Passage. In all treatments enhanced phytoplankton growth was observed compared to non-aerated control bottles. Under elevated CO_2 conditions the nutrient uptake rates and growth rates of cells < 20 µm increased significantly. In the high CO_2 incubations also significantly more POC was accumulated. On the other hand, species composition was not affected by the imposed pCO_2 and no significant differences in [Chl *a*] and *in vivo* fluorescence were observed. Our experiments suggest that elevated pCO_2 stimulates phytoplankton nutrient uptake and ultimately growth under nutrient replete conditions. However, iron and light availability were clearly the controlling factors of phytoplankton physiology in our incubations experiments. In situations where natural iron fertilization occurs, e.g. in coastal waters or by means of upwelling, dust deposition or sea ice melting, increased CO_2 may stimulate phytoplankton physiology and productivity.

INTRODUCTION

The atmospheric concentration of CO_2 has increased from about 280 10^{-6} atm (µatm) since the start of the Industrial Revolution to 390 µatm nowadays and is predicted to rise to 750 µatm or higher by the year 2100 (IPCC 2007). Increases in atmospheric CO_2 by equilibration lead to proportional increase of dissolved CO_2 in seawater, as well as substantial increase of dissolved bicarbonate (HCO_3^-). It is estimated that around 30 % of anthropogenic CO_2 emissions is taken up by the oceans (Le Quéré *et al.* 2009). Increasing concentrations of anthropogenic CO_2 are now observed even in the ocean interior (van Heuven *et al.* 2011), while associated changes (Feely *et al.* 2004) in carbon speciation are described for surface seawaters globally (Olafsson *et al.* 2009, Midorikawa *et al.* 2012).

Phytoplankton represents an important link in the carbon cycle by fixing CO_2 from the surface water, biomass formation and accompanying transfer of carbon to other compartments of the ecosystem. This happens according to the principle of the biological carbon pump, where biomass (carbon and other major and trace elements) are transferred from the surface euphotic zone, down the water column into the ocean interior, thereby effectively removing carbon from the surface biospherical processes for many years to centuries.

Fixation of CO_2 in the surface ocean by phytoplankton also drives CO_2 uptake from the atmosphere. The carbon cycle is closely linked to climate: on one hand, the uptake by the ocean in part regulates the accumulation of this greenhouse gas in the atmosphere, thus controlling global warming; on the other hand, the rate of CO_2 uptake by the ocean is affected by climate change through changes in biogeochemical and physical processes.

The open Southern Ocean is the largest upwelling region of the globe, comprising 20 % of the world oceans. Despite the abundance of major nutrients (N, P, Si, adequate for supporting significant CO_2 fixation), phytoplanktonic primary productivity is limited in large regions of the Southern Ocean. It has been established through bottle and *in situ* Fe enrichment experiments that Fe and light limitation are governing Southern Ocean primary productivity and biological carbon uptake (de Baar *et al.* 1999, Boyd 2002, de Baar *et al.* 2008a).

Elevated concentrations of CO_2 ($[CO_2]$) in seawater also might have a direct effect on phytoplankton physiology (e.g. changes in C uptake due to changes in carbon speciation) resulting in changes in phytoplankton productivity and/or species composition with unknown consequences for the efficiency of the biological carbon pump (Riebesell *et al.* 2007, Beardall *et al.* 2009). It has been demonstrated that increased $[CO_2]$ had taxon-specific effects on marine phytoplankton in the Equatorial Pacific (Tortell *et al.* 2002): high $[CO_2]$ stimulated *Phaeocystis sp.*,

whereas at low [CO_2] diatoms prevailed. Feng *et al.* (2009) investigated the combined effects of [CO_2] and temperature on phytoplankton from the North Atlantic spring bloom, observing a higher coccolithophorid abundance in the "greenhouse" treatment (elevated [CO_2] and temperature). For phytoplankton from the Southern Ocean (specifically, from the Ross Sea) Tortell *et al.* (2008) described a shift in species composition within the same taxon: high [CO_2] favoured larger chain-forming diatoms, while small pennate diatoms dominated under low [CO_2] conditions.

Experiments focussing on the effects of different [CO_2] on natural phytoplankton populations in Antarctic waters nevertheless remain scarce and to the best of our knowledge no experiments have been described for the Atlantic sector of the Southern Ocean. Close to the Antarctic continent phytoplankton can be exposed to [CO_2] as low as 100 µatm (Ross Sea (Sweeney 2003) and up to as high as 400 µatm (surface waters around the Antarctic Peninsula, Rutgers van der Loeff *et al.* 2011). It can therefore be expected that the phytoplankton community is able to adapt to variations in CO_2 (Neven *et al.* 2011).

To assess the response of natural phytoplankton communities to different pCO_2 forcings (190, 380 and 750 µatm) we studied various growth parameters, photosynthetic efficiency, pigment (i.e., phytoplankton species) composition and particulate organic carbon (POC) accumulation in on-board experiments lasting 10 to 14 days. During the experiments, elaborate precautions were taken to maintain the differences in pCO_2 conditions. Our working hypotheses were that increased pCO_2 would stimulate phytoplankton growth through changes in carbon uptake physiology and would cause shifts in phytoplankton species composition. In Chapter 4 results are presented from the same pCO_2 manipulation experiments focusing on the potential of natural Southern Ocean phytoplankton communities to adapt their DIC acquisition.

MATERIAL AND METHODS

Incubation experiments were done on board of RV Polarstern during expedition ANT XXIV-3 (10 February - 17 April 2008; (Fahrbach & de Baar 2010)) in the Atlantic sector of the Southern Ocean. An overview of the sampling locations is given in Figure 3.1. Experiment A was started at the Polar Front on the Prime Meridian, Experiment B and C on the Prime Meridian close to the ice edge, Experiment D and E in the Weddell Sea and experiment F in the Drake Passage close to King George Island.

Sampling and experimental design

Seawater was taken from an ultra clean, all-titanium CTD system equipped with 24 GO-FLO sampling bottles (de Baar *et al.* 2008b). Samples were collected at the depth of the maximum chlorophyll a (Chl *a*) concentration (Table 3.1), determined with a Chelsea fluorescence meter that was mounted on the CTD frame. Once retrieved on board, unfiltered water from the GO-FLO sampling bottles was carefully dispensed into 10 l (Nalgene®) polycarbonate carboys. At t = 0, 4 and 7 days, 3.6 nmol l^{-1} Fe was added to all incubation vessels in order to prevent iron limitation from confounding CO_2-treatment effects. The partial

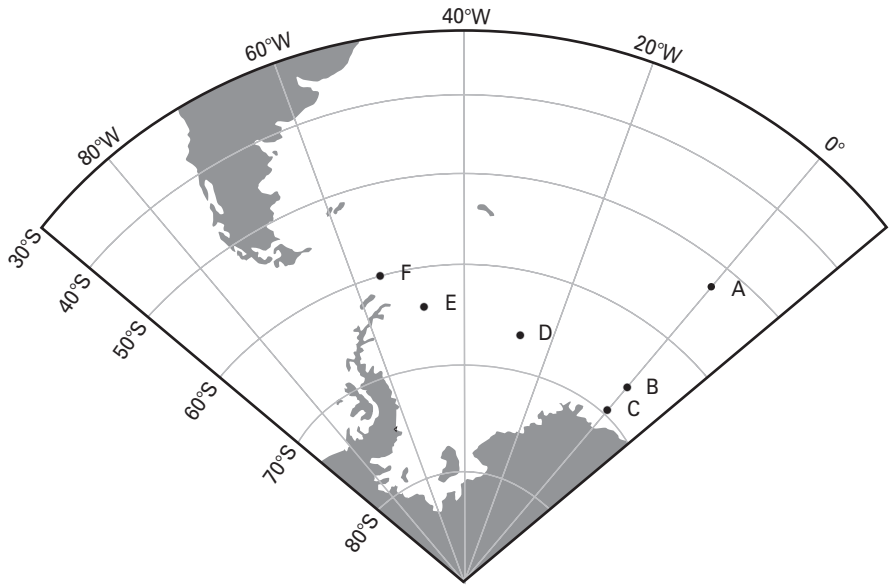


Figure 3.1. Chart of the study area. Black dots represent locations where water for the experiments (A-F) was collected.

pressure of CO_2 was manipulated and maintained by gentle aeration of the bottles with gas streams of different CO_2 concentrations (CO_2-air mixture, LindeGas, The Netherlands). To test whether aeration would cause adverse sheer stress for the phytoplankton, non-aerated control bottles were included in all experiments. All treatments, i.e. 190, 380, 750 µatm pCO_2, respectively mimicking conditions encountered during the last glacial maximum, the present date and future atmospheric CO_2 conditions, and the non-aerated control were done in duplicate. All incubations were done at 4 °C inside a laboratory container, with a 16:8 h light:dark regime and an average light intensity of 50 µmol photons m^{-2} s^{-1}. The incubations typically lasted 10 to 14 days.

DIC measurements

Samples for measurements of DIC and total alkalinity (A_T) were collected and analysed in general accordance with Dickson *et al.* (2007). For collection of samples from the cultures, 250 ml stoppered, borosilicate-glass sample bottles (Schott Duran®) were rinsed twice to reach culture temperature and filled with sample, which was pushed out of the culture vessels by slightly applying overpressure to the headspace. Samples were poisoned with 100 µl of a saturated solution of $HgCl_2$ and stored dark and cold. Samples were analysed on two VINDTA 3C instruments (MARIANDA, Kiel, Germany) generally within 6 h, and in all cases within 48 h after sampling. In order to set the measurement accuracy, certified reference material (made available by Prof. A. Dickson, Scripps Institute of Oceanography, USA; (Dickson 2010)) was analysed at least 3 times per day.

The speciation of carbon was calculated from A_T and DIC using CO2SYS (van Heuven *et al.* 2009; updated in 2011), using CO_2 acidity constants of Mehrbach *et al.* (1973) as refit by Dickson & Millero (1987), the CO_2 solubility coefficient of Weiss (1974), the borate acidity constant of Dickson (1990) and the SO_4^{2-} dissociation constant of Dickson *et al.* (1990). In the calculations of the carbon speciation the measured values of salinity and major nutrient concentrations were incorporated.

Pulse amplitude modulation fluorometry

Daily assessments were made of *in vivo* chlorophyll-fluorescence (F_0), maximum chlorophyll-fluorescence (F_m) as well as of the photosynthetic efficiency (F_v/F_m, where F_v is F_m-F_0) of the phytoplanktonic community using a Pulse Amplitude Modulation (PAM) fluorimeter (PHYTOPAM, Walz™, Germany) following the method of Schreiber *et al.* (1986). Samples were kept cold and dark adapted for at least 15 minutes before the fluorescence measurements.

Photopigments

Samples for photosynthetic pigments were collected at least 3 times during the experiment. After filtration (< 0.2 kPa) of known sample volumes on 25 mm Whatman™ GF/C filters, filters were frozen instantaneously in liquid nitrogen and stored at -80 °C. Samples were analysed by HPLC in the home laboratory using a C8 separation column according to Zapata *et al.* (2000).

Particulate organic carbon

To determine POC, known volumes of sample were filtered over precombusted Whatman GF/F glass-fibre filters (13 mm), snap-frozen in liquid nitrogen and stored at -80 °C until further processing. At the home laboratory filters were kept in an atmosphere of fuming HCl for 4 h to remove DIC and subsequently dried overnight. Filters were then packed in tin capsules (Elemental Microanalysis Ltd., UK) and analysed using an automated CHNS analyser (automated element analysis, EA 1110, Interscience, New York, USA).

Nutrients - N, P, Si

Concentrations of dissolved nitrate and nitrite (taken together as N), phosphate (P) and silicate (Si) were determined on board on a daily basis. Samples were collected in high-density polyethylene sample bottles, filtered through 0.2 µm filter cartridges (Sartobran-300, Sartorius) and stored in the dark at 4 °C (N and P) or at -20 °C (Si) in polyethylene (PonyVials) cups. Samples were analysed at the end of each experiment to minimize inaccuracies due to instrument drift. Nutrient concentrations were measured spectrophotometrically after Grasshoff & Ehrhardt (1983) using a Traacs AutoAnalyzer 800+ (Bran & Luebbe, Germany) with a precision of 0.03 µmol l^{-1} for P, 0.16 µmol l^{-1} for N and 0.08 µmol l^{-1} for Si (n=1000). Specific nutrient uptake rates were calculated by fitting the nutrients taken up in time with a logistic function:

$$Y = \frac{Y_{max}}{1 + B \times c^{-k!}}$$

where k is the maximum uptake rate and B is a constant. For each experiment the maximum possible uptake (Y_{max}) was set to be the concentration at the start of the experiments.

Cell numbers counted by flow cytometry

Abundance of phytoplankton < 20 µm in the incubation bottles was measured on board with a Coulter Epics XL-MCL flow cytometer on a daily basis. Counts were triggered on red autofluorescence (FL4), measured at > 610 nm. Growth

Table 3.1. Overview of sampling locations for the experiments A-F and their chemical characteristics: Starting dates, geographical positions, sampling depths (m), temperatures (°C), salinity (psu), initial concentrations of P (phosphate), Si (silicate) and N (nitrate + nitrite) (all in µmol kg^{-1}), dissolved Fe (in nmol l^{-1}) and the CO_2 system speciation (DIC and A_T both in µmol kg^{-1}).

	Sampling depth (m)	Temperature (°C)	Salinity (psu)	N (µmol kg^{-1})	P (µmol kg^{-1})	Si (µmol kg^{-1})	dFe (nmol l^{-1})	DIC (µmol kg^{-1})	A_T (µmol kg^{-1})
Experiment A 20 Feb 2008, 9 d									
52°99'S, 0°03'W	73.6	1.2	33.8	26.1	1.75	35.6	0.25	2163	2294
Experiment B 09 Mar 2008, 10 d									
66°50'S, 0°00'W	40.5	-1.7	34.3	29.8	2.09	62.7	0.17	2218	2328
Experiment C 12 Mar 2008, 10 d									
69°40'S, 0°10'E	20.0	-1.5	34.0	24.5	1.85	53.9	0.22	2170	2316
Experiment D 19 Mar 2008, 10 d									
66°59'S, 27°35'E	60.7	-1.6	34.4	28.9	2.00	77.1	0.10	2228	2352
Experiment E 25 Mar 2008, 14 d									
64°05'S, 48°26'E	45.5	-1.6	34.4	30.7	2.11	85.9	0.07	2231	2353
Experiment F 2 Apr 2008, 10 d									
60°10'S, 55°28'E	65.1	0.7	34.2	28.5	2.00	69.1	1.13	2203	2326

rates of the phytoplankton population were calculated by fitting a logistic growth function to the data.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software version 4.03 (GraphPad Software, San Diego, CA, USA) by means of one-way analysis of variance (ANOVA) with repeated measures, followed by either Tukey's multiple comparison test to compare all pairs of columns and/or by post test for linear trend.

RESULTS

General description of the phytoplankton community at the beginning of the experiments

Initial physical, chemical and biological conditions are presented in Table 3.1. Experiment A was started north of 60 °S with seawater temperature > 0 °C and typical open ocean major nutrients (N, P, Si) concentrations (Knox 2007). Experiments B - E were started close to the Antarctic continent with seawater temperature < 0 °C and major nutrient concentrations typical for 'high nutrient low chlorophyll' regions. Experiment F was started close to King George Island. There, initial seawater temperature at sampling depth was > 0 °C and major nutrient concentrations were still high.

The accuracy of the [DIC] and A_T measurements is estimated to be 5 and 6 μmol kg^{-1}, respectively. Values for DIC and A_T at the beginning of the experiments A - F were between 2163-2231 μmol kg^{-1} for DIC, and 2294-2353 μmol kg^{-1} for A_T, respectively. A more elaborate description of the carbonate system during this expedition can be found in van Heuven *et al.* (2011).

Initial concentrations of dissolved Fe were low for experiment A - E, ranging from 0.07 to 0.22 nmol l^{-1} (Klunder *et al.* 2011). Experiment F differed from the other experiments, because dissolved Fe concentrations were relatively high (1.13 nmol l^{-1}).

Manipulation of the DIC system in the incubations

Aeration with the CO_2-air mixtures significantly changed average [CO_2] (one-way ANOVA with repeated measures, $p < 0.0001$) and DIC (one-way ANOVA with repeated measures, $p = 0.0002$) in the incubations (Figures 3.2A and B) and resulted for both parameters in the following ascending order: 190 μatm < 380 μatm < non-aerated control < 750 μatm. As expected, only a small variation in A_T was measured for all incubations (Figure 3.2C). The small standard deviations of A_T (< 5 %) reflects the robustness of the pCO_2 treatments.

Although the three desired pCO_2 values of 190, 380 and 750 μatm were not reached exactly, distinct differences in DIC and [CO_2] between the pCO_2 treatments were visible 24 h after the start of the experiment. In the course of the experiments a gradual decrease in [CO_2] was observed for all pCO_2 treatments.

Maximal quantum yield of photosystem II (F_v/F_m)

The maximal quantum yield of photosystem II (F_v/F_m), measured as an indicator for photosynthetic efficiency of phytoplankton, generally increased during the experiments (Figure 3.3) with the exception of experiment F. At the beginning of

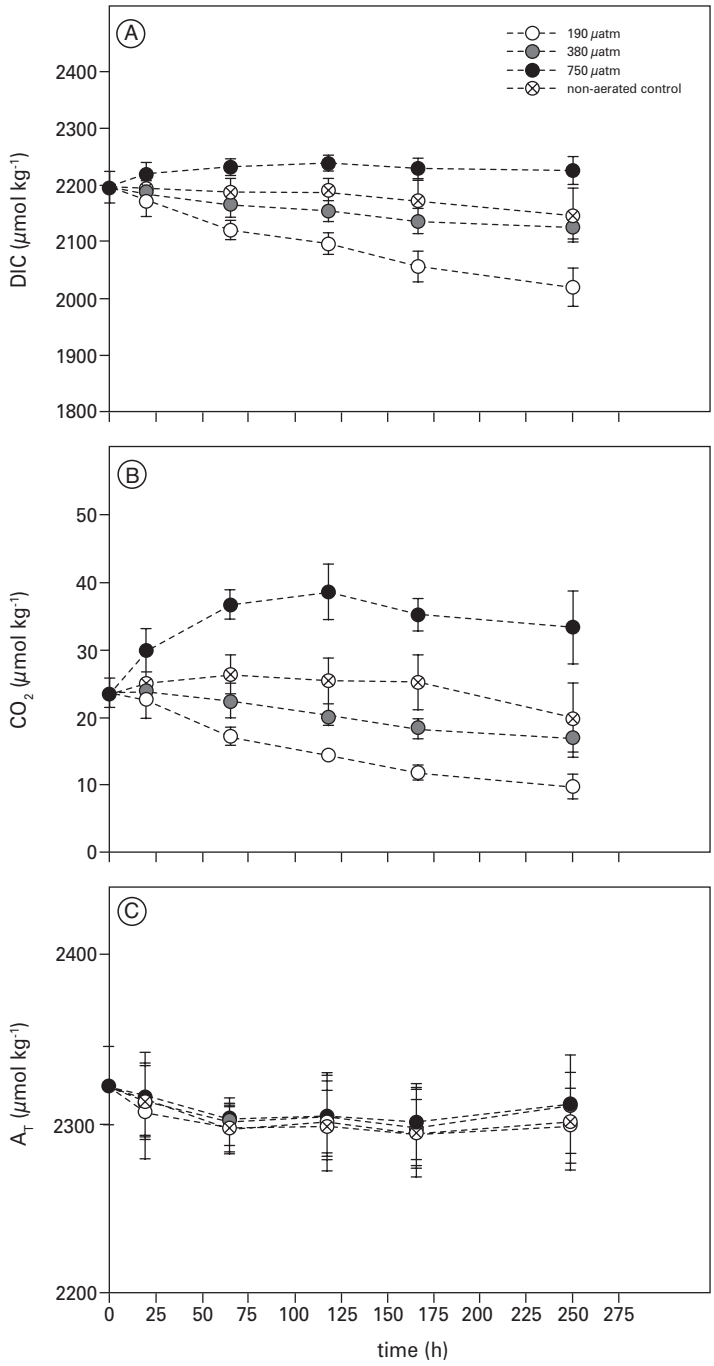


Figure 3.2. Properties of the DIC system (in µmol kg^{-1}) over time in the experiments (A - F). (A) Average DIC, (B) Average CO_2 concentrations, (C) Average total alkalinity (A_T).

the experiments A - E, F_v/F_m averaged 0.44 ± 0.07, increased gradually to 0.62 ± 0.06 at $T_{1/2}$ and decreased again to 0.51 ± 0.09 at the end of the experiments. F_v/F_m remained constant in experiment F (0.54 at t = 0, 0.54 ± 0.03 at $T_{1/2}$ and 0.56 ± 0.03 at T_{end}). No consistent differences between the different pCO_2 treatments in terms of F_v/F_m were observed in any of the experiments.

Growth rates derived from* in vivo *chlorophyll-fluorescence

Chlorophyll fluorescence after dark adaptation (F_0), which can be regarded as estimation for chlorophyll a biomass, increased during the experiments (data not shown). However, no significant trend between the different pCO_2 treatments was found in growth rates calculated with a logistic function from the increase in F_0 fluorescence in time (Table 3.2A).

Growth rates derived from cell numbers determined by flow cytometry

Cell numbers of cells < 20 µm increased in the course of the experiments (Figure 3.4). Growth rates were calculated by fitting a logistic growth function to cell numbers determined by flow cytometry (Table 3.2B). Growth rates differed significantly between pCO_2 treatments (one-way ANOVA with repeated measures, p = 0.0485), more specifically: cells in the 190 µatm treatment grew slower than cells in the 750 µatm treatment (Tukey's multiple comparison test, p = *) and a linear trend following the order 190 < 380 < 750 µatm was observed (Post test for linear trend, p = 0.0164).

Table 3.2. (A) Growth rates (d^{-1}) of phytoplankton in the incubation bottles derived from F_0 determined by pulse amplitude modulation fluorometry. (B) Growth rates (d^{-1}) of phytoplankton in the incubation bottles derived from cell counts (< 20 µm) determined by flow cytometry.

(A)	CO_2 treatment (µatm)	A	B	C	D	E	F	Average ± SD
	190	0.38	0.59	0.69	0.55	0.43	0.51	0.53 ± 0.11
	380	0.44	0.54	0.70	0.51	0.49	0.55	0.54 ± 0.09
	750	0.44	0.53	0.53	0.58	0.52	0.52	0.52 ± 0.05
	Non-aerated control	0.46	0.45	0.48	0.57	0.46	0.32	0.49 ± 0.05
(B)	CO_2 treatment (µatm)	A	B	C	D	E	F	Average ± SD
	190	0.27	0.16	0.28	0.50	0.26	0.24	0.28 ± 0.11
	380	0.28	0.18	0.33	0.48	0.29	0.26	0.31 ± 0.10
	750	0.38	0.22	0.32	0.49	0.31	0.25	0.33 ± 0.09
	Non-aerated control	0.32	0.21	0.22	0.35	0.23	0.15	0.25 ± 0.07

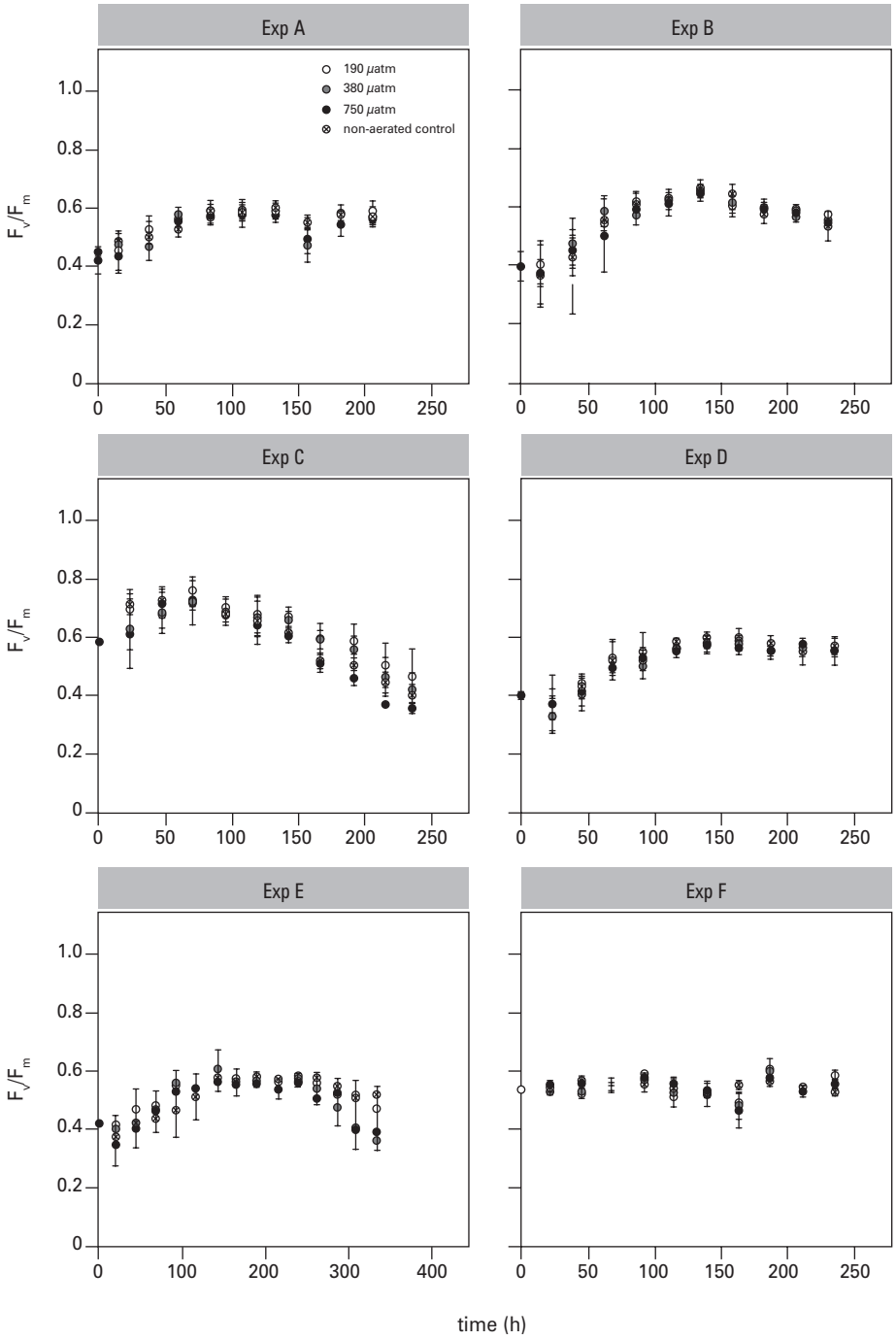


Figure 3.3. Maximal quantum yield of photosystem II (F_v/F_m) of dark-adapted phytoplankton per experiment (A - F).

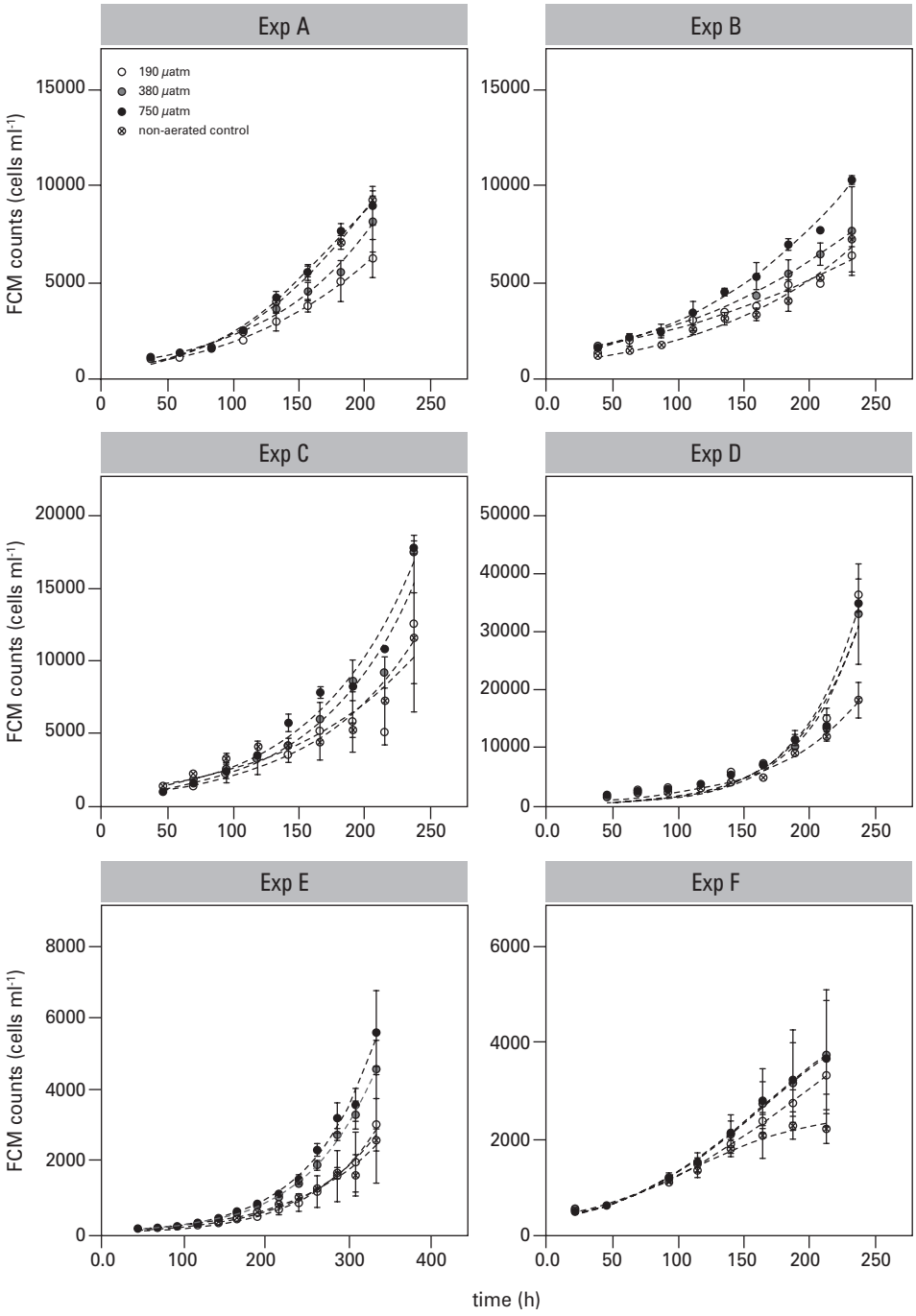


Figure 3.4. Cell numbers (in cells ml^{-1}) per experiment (A - F).

Nutrient uptake rates - N, P, Si

Typically, nutrient uptake in the incubations was low in the beginning and increased after a short lag phase towards the end (Figures 3.5, 3.6 and 3.7). Aerated incubations (190, 380 and 750 µatm) took up significantly more nutrients than the non-aerated control treatment (Table 3.3, one-way ANOVA with repeated measures, $p < 0.05$). Nitrate was depleted in the 750 and 380 µatm incubations at the end of the experiments C and E. In the same incubations (and in the non-aerated control incubation in experiment C) P was reduced to < 0.5 µM l^{-1}. Silicate was never depleted in any of the experiments.

In general, there was a significant linear increase in the uptake of all nutrients from low to high pCO_2 (one-way ANOVA with repeated measures, $p < 0.05$ for N, P, Si). Significant differences between the pCO_2 treatments were observed in the uptake rates of Si and P (one-way ANOVA with repeated measures, $p = 0.0002$ and $p = 0.005$, respectively). Although not significant, the same trend was observed for N uptake (one-way ANOVA with repeated measures, $p = 0.0627$). Specifically, Si uptake differed significantly between all treatments. P uptake differed between all treatments with the exception of 380 µatm compared to 750 µatm. N uptake differed significantly between non-aerated control and the 750 µatm treatment.

Table 3.3. (A) Specific N uptake rates (d^{-1}) for the different experiments. (B) Specific P uptake rates (d^{-1}) for the different experiments. (C) Specific Si uptake rates (d^{-1}) for the different experiments.

(A) N	CO_2 treatment (µatm)	A	B	C	D	E	F	Average ± SD
	190	0.54	0.61	0.65	0.71	0.47	0.69	0.61 ± 0.09
	380	0.63	0.68	0.80	0.64	0.76	0.69	0.70 ± 0.06
	750	0.63	0.64	0.84	0.68	0.78	0.76	0.72 ± 0.08
	Non-aerated control	0.58	0.46	0.69	0.52	0.57	0.55	0.56 ± 0.07
(B) P	CO_2 treatment (µatm)	A	B	C	D	E	F	Average ± SD
	190	0.43	0.56	0.85	0.67	0.47	0.60	0.59 ± 0.16
	380	0.58	0.69	1.02	0.65	0.68	0.61	0.71 ± 0.16
	750	0.52	0.72	1.04	0.71	0.71	0.66	0.73 ± 0.17
	Non-aerated control	0.60	0.52	0.72	0.50	0.56	0.42	0.55 ± 0.10
(C) Si	CO_2 treatment (µatm)	A	B	C	D	E	F	Average ± SD
	190	0.49	0.47	0.65	0.49	0.31	0.39	0.47 ± 0.11
	380	0.52	0.51	0.69	0.52	0.40	0.45	0.51 ± 0.10
	750	0.55	0.56	0.77	0.56	0.41	0.57	0.57 ± 0.12
	Non-aerated control	0.47	0.36	0.66	0.49	0.38	0.42	0.47 ± 0.12

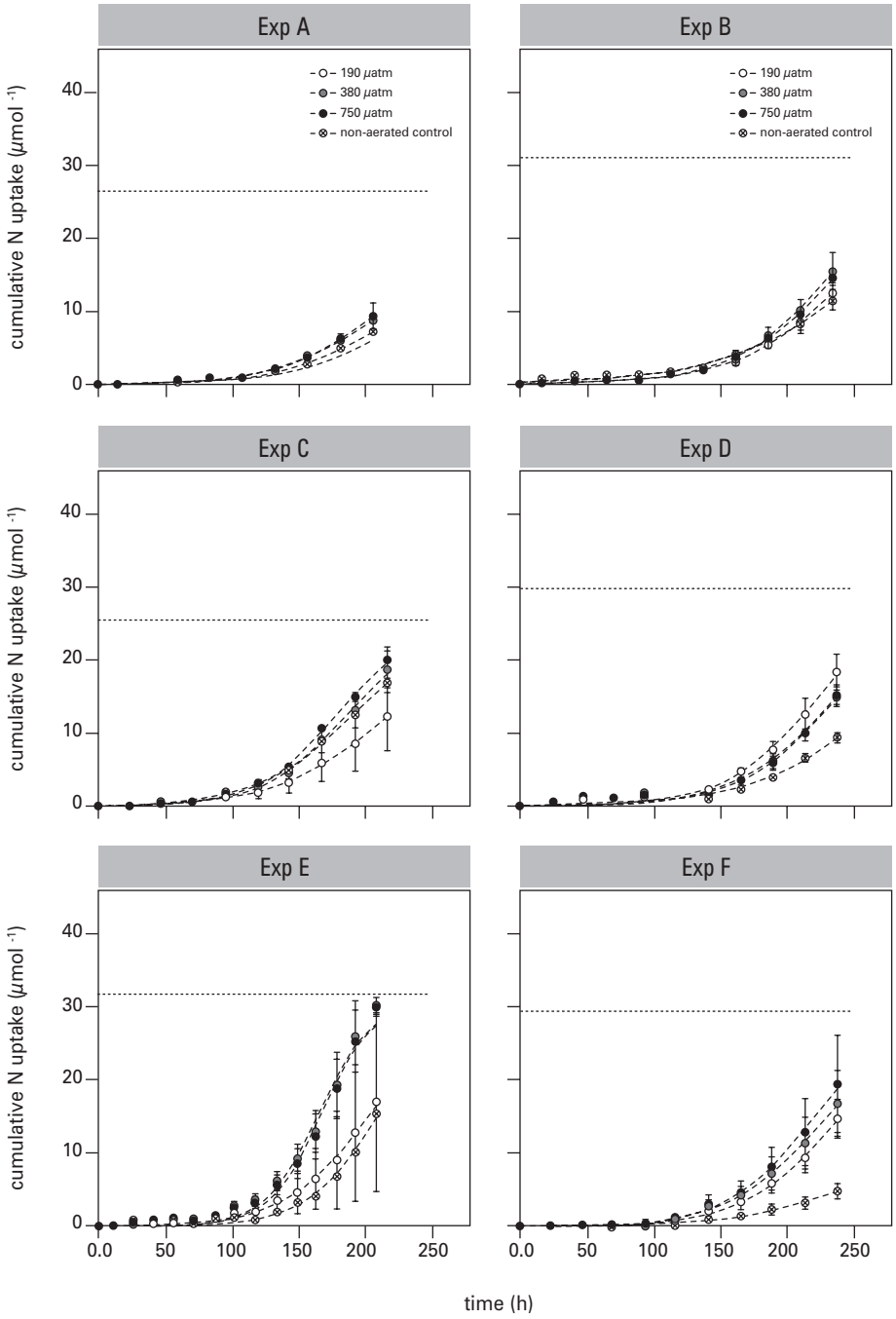


Figure 3.5. Cumulative N uptake (in µM l^{-1}) per experiment (A - F). The horizontal line represents the initial N concentration.

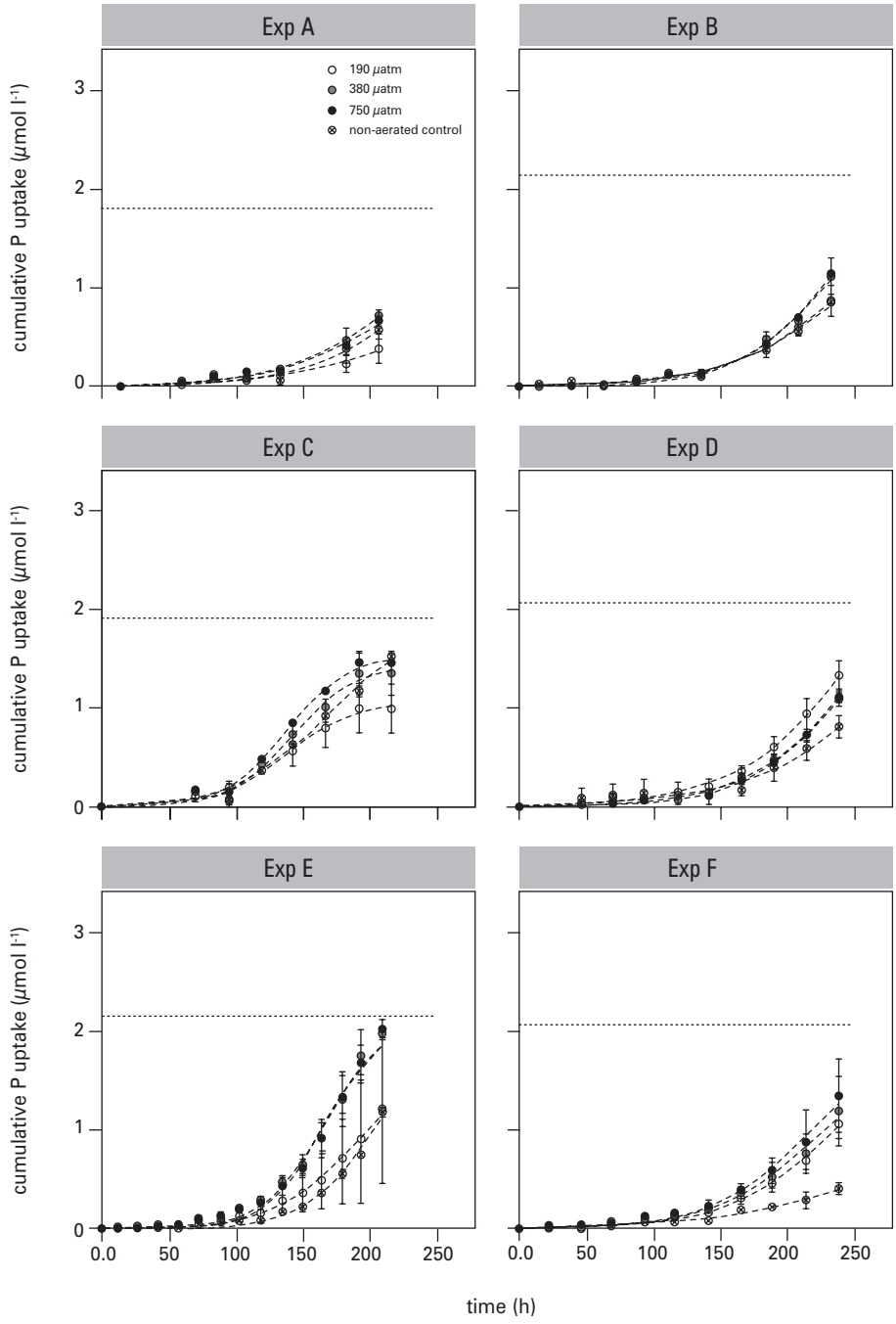


Figure 3.6. Cumulative P uptake (in µM l^{-1}) per experiment (A - F). The horizontal line represents the initial P concentration.

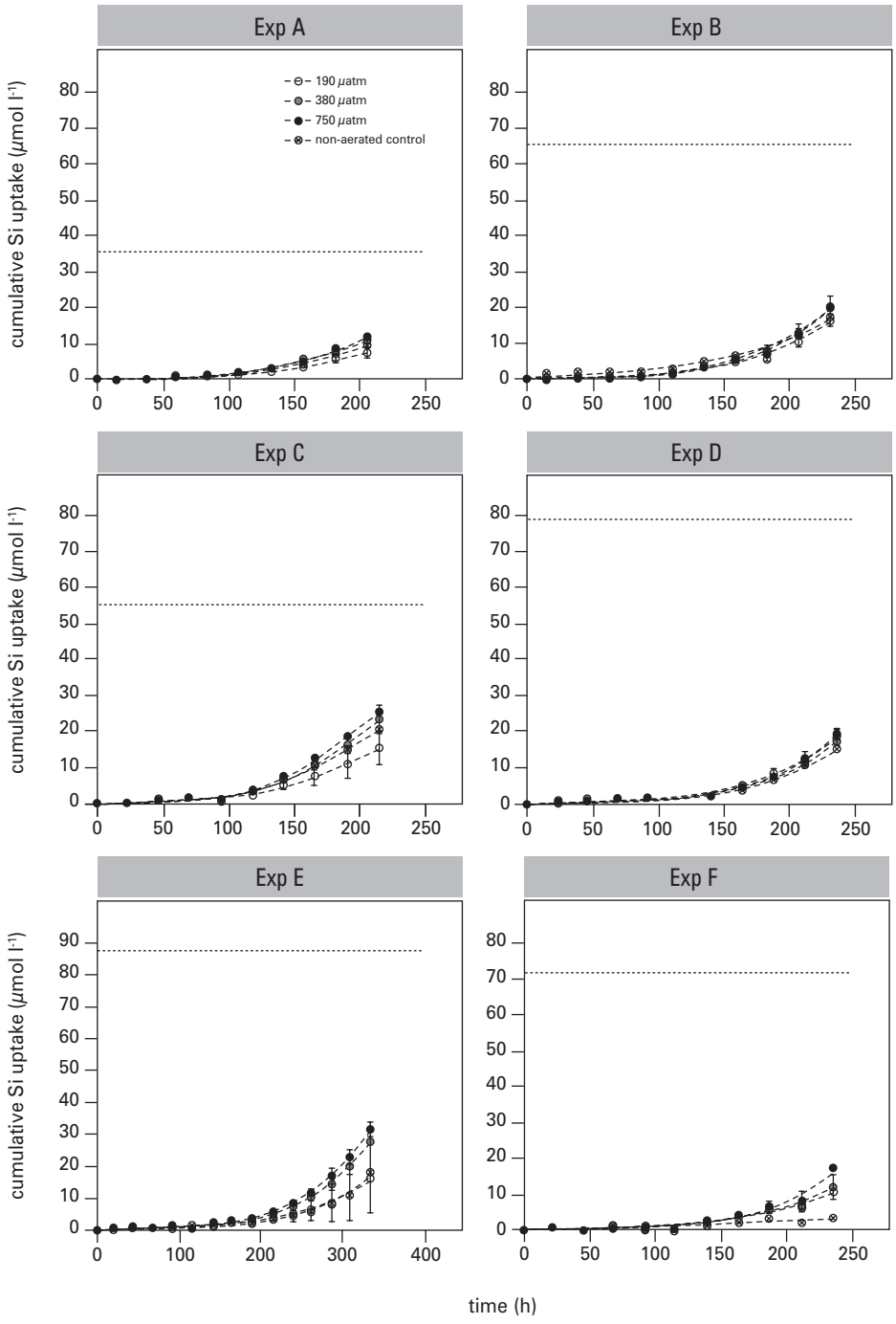


Figure 3.7. Cumulative Si uptake (in µM l^{-1}) per experiment (A - F). The horizontal line represents the initial Si concentration.

***POC, Chl* a *and POC:Chl* a**

Analysis of samples for POC revealed significant differences between the pCO_2 treatments (one-way ANOVA with repeated measures, p = 0.0001, Figure 3.8A). At the end of the experiments phytoplankton accumulated significantly more POC under high pCO_2 than under low pCO_2 (Post test for linear trend, p = 0.0004). Phytoplankton incubated at 750 µatm pCO_2 produced significantly more POC than at 190 µatm (p = ***) and at 380 µatm (p = *). POC accumulation in the 380 µatm treatment did not differ from the 190 µatm (all Tukey's multiple comparison test) treatment. The non-aerated control accumulated significantly less POC than all the other treatments (p = ** for the 190 µatm versus the non-aerated control and p = *** for the 380 and 750 µatm versus the non-aerated control, Tukey's multiple comparison test). At $T_{1/2}$ no differences in the accumulation of POC were present between the treatments (one-way ANOVA with repeated measures, p = 0.117).

Average Chl *a* increased during the experiments after a short lag phase (Figure 3.8B). No significant differences between the treatments were observed, but non-aerated control bottles grew significantly slower than 380 and 750 µatm cultures (one-way ANOVA with repeated measures, p = 0.006).

A significant decrease of POC:Chl *a* over time was observed (two-way ANOVA with repeated measures, p < 0.0001; Figure 3.8C). No trend between the different pCO_2 regimes was found.

Species composition derived from photopigments

In general, the composition of the most abundant photopigments changed during the time course of the experiments (Figure 3.9). Chlorophyll a (Chl *a*) was the most abundant pigment in all experiments accounting for 28 - 50 % of total pigment content. Chlorophyll b (Chl b) was only encountered in the beginning of experiment E and F. (1 - 5 %). Chlorophyll c2 (Chl c2) was present in all experiments in small amounts (0.5 - 8 %) and decreased during the experiments (with the exception of experiment A during which Chl c2 increased from 16 % to approximately 25 %). Chlorophyll c3 (Chl c3) decreased in all experiments from approximately 14.6 ± 5.2 % to 6.4 ± 0.4 %. Diadinoxanthin (diadino) was present in all samples in relatively small amounts (5 - 7 %). The pigment 19'-hexanoyloxyfucoxanthin (hexa) was initially present in all experiments and decreased to zero towards the end. Fucoxanthin (fuco) increased from 26.1 ± 4.7 % to more than 1/3 of the total pigment content at the end of the experiments. The pigment 19'-butanoyloxyfucoxanthin (19but) was usually present in small amounts (3.6 ± 3.4 %) at the beginning and hardly detectable at the end of the experiments. No significant differences in pigment composition between the different pCO_2 treatments were found.

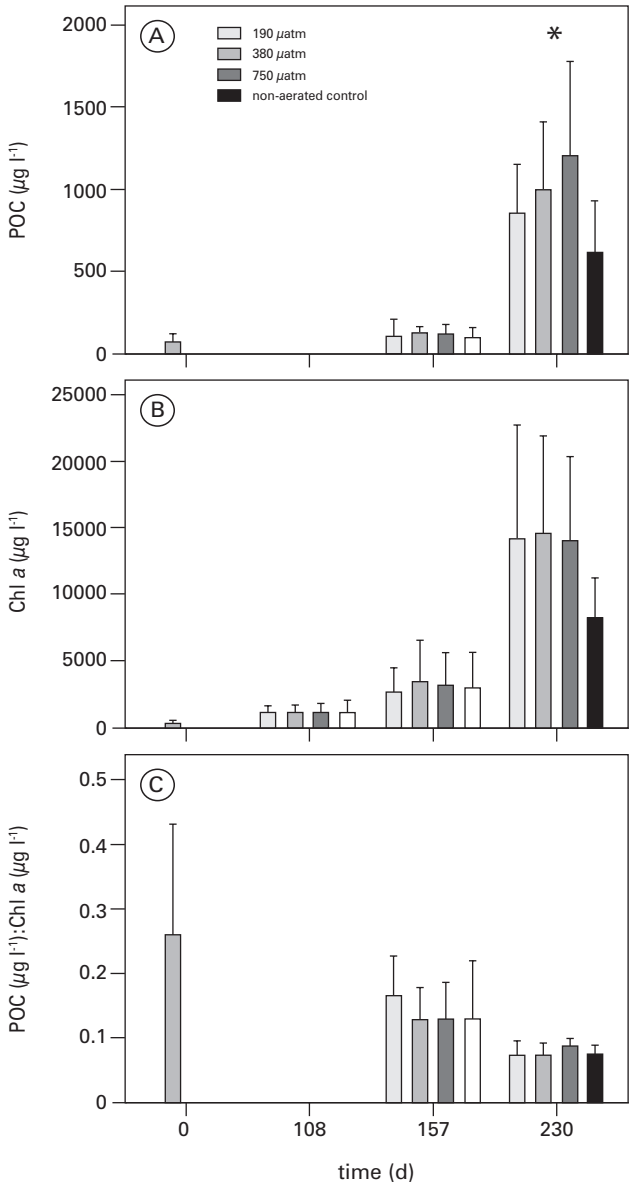
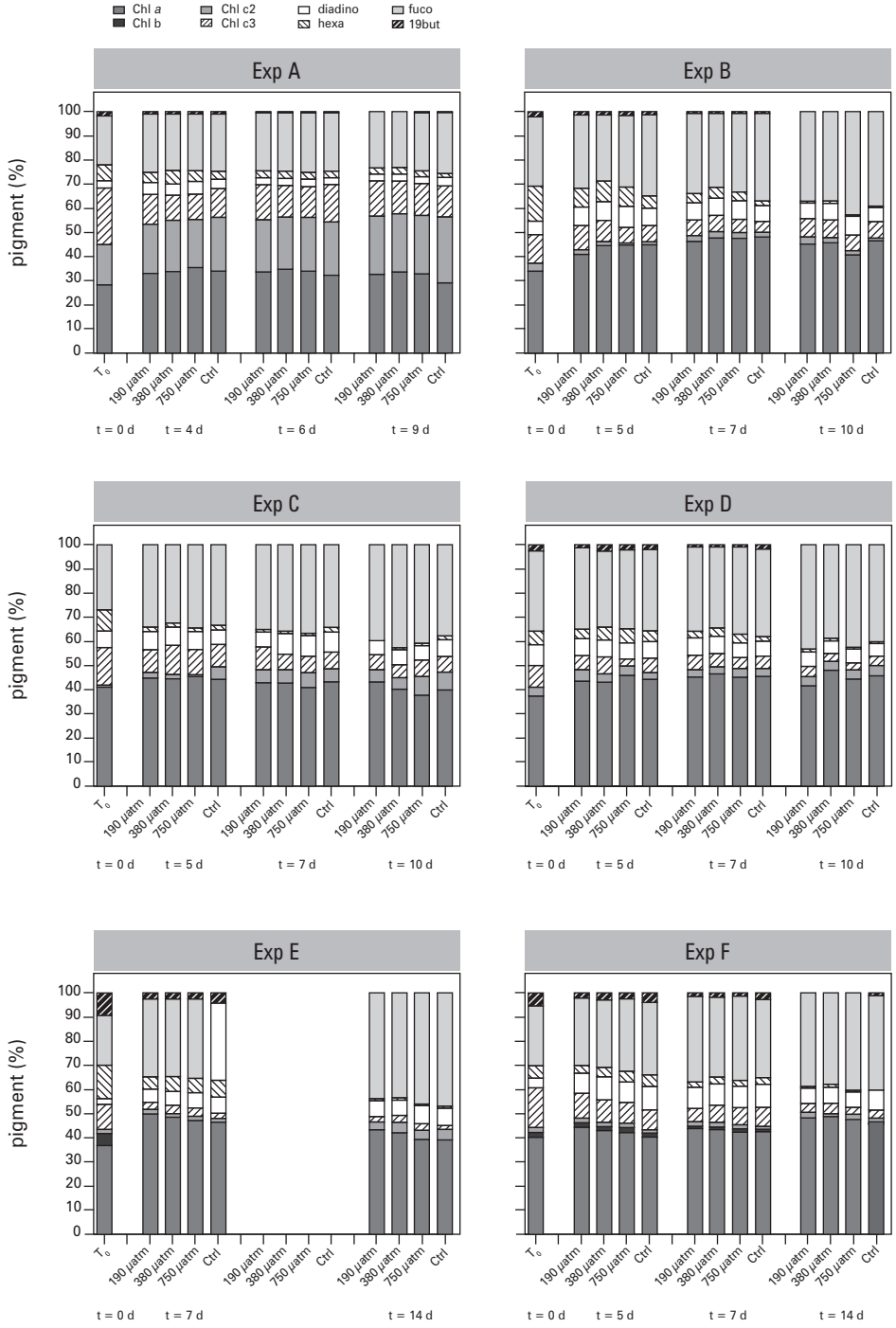


Figure 3.8. Chl *a*, POC and POC:Chl *a* in the experiments A-F. (A) Average POC (µg l^{-1}), (B) Average Chl *a* (µg l^{-1}), (C) Average POC (µg l^{-1}):Chl *a* (µg l^{-1}).

Figure 3.9. Overview of the relative concentrations of the most abundant pigments in the experiments A - F (Chlorophyll a (Chl *a*), Chlorophyll b (Chl b), Chlorophyll c2 (Chl c2), Chlorophyll c3 (Chl c3), diadinoxanthin (diadino), 19'-hexanoylfucoxanthin (hexa), fucoxanthin (fuco), 19'-butanoylfucoxanthin (19but)) expressed as percentage of the total pigment concentration. ▶


Chl a
Chl b
Chl c2
Chl c3
diadino
hexa
fuco
19but
Exp A
Exp B
Exp C
Exp D
Exp E
Exp F
pigment (%)
T0
190 µatm
380 µatm
750 µatm
Ctrl
t = 0 d
t = 4 d
t = 6 d
t = 9 d
t = 5 d
t = 7 d
t = 10 d
t = 14 d
time (h)

DISCUSSION

The Southern Ocean is recognized for its large influence on the global marine carbon cycle, including its regulating effects on the atmospheric CO_2 concentration by means of the biological pump (Sabine *et al.* 2004, van Heuven *et al.* 2011). Two factors that control phytoplankton growth in the Southern Ocean have been identified in the past: iron (Fe) and light. Fe limitation causes high nutrient low chlorophyll (HNLC) conditions (Boyd 2002) in the open Southern Ocean, whereas natural Fe fertilization can result in localized phytoplankton bloom formation (de Baar *et al.* 1995, Blain *et al.* 2007). Deep mixing can impose light limitation on phytoplankton. In contrast, excessive light exposure at the surface can cause photoinhibition of phytoplankton (Alderkamp *et al.* 2010). The combination of these different processes shapes the ambient phytoplanktonic community.

To investigate whether natural variability in pCO_2 also influences primary productivity in the Southern Ocean, we performed pCO_2 manipulation experiments with local, natural phytoplankton communities. Overall, we observed an increased productivity with increasing pCO_2 but this was not evident in all parameters. Understanding the influence of pCO_2 on phytoplanktonic ecology and physiology is not only of fundamental interest, but becomes increasingly important in the light of rising atmospheric and oceanic pCO_2.

Limitations to changing the DIC system in on-board incubations

The different pCO_2 conditions in the experimental set-up could well be established and maintained (Figure 3.2). The concentrations of DIC and CO_2 differed significantly between the treatments, while A_T remained unchanged demonstrating the appropriateness of culture manipulations. The $[CO_2]$ gradually decreased during all experiments indicating (at least for the highest pCO_2 treatment) that aeration was not fast enough to compensate for phytoplanktonic carbon uptake.

The initial nutrient conditions were high, as is characteristic for HNLC Southern Ocean waters. Dissolved P, Si as well as N concentrations were available well above half-saturation values for the growth of e.g. diatoms (Sarthou *et al.* 2005). In a preliminary experiment it proved impossible to maintain cultures Fe limited when aerated in our set up. Therefore we choose to add Fe in known quantities, so that conditions were comparable for all experiments and incubations vessels and pCO_2 was the only variable that differed between the incubation vessels. This approach resulted in typical grow-out conditions, in which growth was determined by light and $[CO_2]$.

Several methods have been applied to manipulate the carbonate system in seawater (for a comprehensive consideration of the advantages and disadvantages

please see Chapter 2 of Riebesell *et al.* 2010). We chose to manipulate pCO_2 by aeration because this method reproduces all parameters of the carbonate systems accurately for future high atmospheric pCO_2 conditions in an open system. However, negative effects of continuous aeration due to turbulence have been reported (Shi *et al.* 2009). In order to test potential harmful effects of continuous aeration, control bottles without aeration were added in every experiment. Although DIC and pCO_2 in the control bottles were relatively high (compared to the 380 µatm pCO_2 treatment), all measured parameters indicated that non-aerated control cultures grew significantly slower than 380 and 750 µatm aerated cultures. This suggests that another factor than pCO_2 - presumably light - was limiting growth. In the aerated incubations better mixing might have improved light conditions, reduced the unstirred layer or negatively affected micro-zooplankton grazing activity.

Why do growth rates derived from different parameters differ from each other?

Growth rates derived from different parameters gave different results, demonstrating that care needs to be taken when evaluating the outcome of these experiments. Average growth rates derived from flow cytometer cell counts ranged from 0.25 - 0.33 d^{-1}, while average growth rates derived from F_0 fluorescence ranged from 0.49 - 0.54 d^{-1} (Table 3.2A). Average specific nutrient uptake rates even ranged from 0.47 - 0.73 d^{-1} (Table 3.3). The observed differences in growth rates can be partially explained by the different methods employed. Flow cytometry is restricted to cells < 20 µm, whereas PAM fluorometry and nutrient uptake rates are representative of the whole community. Growth rates derived from F_0 fluorescence data were considerably higher than those derived from flow cytometry indicating that larger cells grew faster in the incubations. This idea is supported by the fact that nutrient uptake rates were approximately twice as high as growth rates derived from flow cytometry. Veldhuis & Timmermans (2007) have shown that larger cells increased during bottle incubations in experiments with natural phytoplankton assemblages from the Southern Ocean. Based on nutrient uptake and growth rates derived from F_0 measurements we speculate, that this also has happened in our incubations.

Effect of culturing conditions on phytoplankton growth parameters

The phytoplankton community at T_0 differed from the phytoplankton community in the incubations in terms of physiology, growth rates and species composition.

The *in vivo* chlorophyll fluorescence data revealed that other factors than $[CO_2]$ affected phytoplanktonic physiology and consequently growth and species

composition. Initial values for the maximum quantum yield of photosystem II (F_v/F_m), which can be used as an indicator for photosynthetic efficiency, were 0.44 ± 0.07. These values match well with the F_v/F_m values measured by Alderkamp *et al.* (2010) during the same cruise, who reported values of 0.40 - 0.47 for surface samples along Greenwich Meridian. In the course of the experiments F_v/F_m increased in 5 of the 6 experiments (to 0.62 ± 0.06 at $T_{1/2}$) suggesting that cells had been stressed under ambient conditions. An increase in F_v/F_m is often observed after nutrient limitation is alleviated and even before growth rates increase (Boyd & Abraham 2001, Holeton *et al.* 2005, Behrenfeld *et al.* 2006). As all major nutrients were present in ample supply we suggest that the immediate increase in F_v/F_m is related to the addition of Fe (see also Chapter 4). This view is also supported by the fact that the only experiment were F_v/F_m did not increase was experiment F, for which relatively high *in situ* dFe concentration were measured. At the end of the experiments F_v/F_m decreased to 0.51 ± 0.09. Nitrogen and phosphorus uptake data show that both nutrients were depleted at the end of the experiments C and E in most treatments suggesting that the decrease in F_v/F_m is a consequence of N and P depletion.

Effect of culturing conditions on phytoplankton species composition

In general, pigment ratios of marker pigments are used to visualize changes in species composition. However, several studies have shown that environmental factors such as iron and light availability can considerably affect these ratios in different ways (van Leeuwe & Stefels 1998, Schoemann *et al.* 2005, van Leeuwe & Stefels 2007, Feng *et al.* 2010). As a result, environmental factors and changes in species composition might amplify or cancel each other out. Therefore data on marker pigment ratios need to be interpreted with caution. An overview of how changes in species composition and changes of irradiance and Fe supply affect commonly used marker pigment ratios is given in Table 3.5 to facilitate interpretation of these ratios.

In brief, iron is known to stimulate Chl *a* (Geider & MacIntyre 2002, de Baar *et al.* 2005), fucoxanthin and Chl c3 biosynthesis (Feng *et al.* 2010). However, relative to Chl *a*, Chl c3 increases less (Di Tullio *et al.* 2007, van Leeuwe & Stefels 2007). Hexa has been shown to decrease upon Fe addition (van Leeuwe & Stefels 2007).

Low light has been shown to result in an increase of the light harvesting pigments Chl *a*, 19'-hexanoyloxyfucoxanthin and fucoxanthin (van Leeuwe & Stefels 1998). Feng *et al.* (2010) observed a faster increase of fucoxanthin relative to 19'-hexanoyloxyfucoxanthin at low light.

Fucoxanthin and Chl *a* are common pigments that occur in almost all phytoplanktonic groups (Jeffrey 1997). On the other hand, the pigment 19'-hexanoyloxyfucoxanthin is characteristic for Prymnesiophyceae such as *Phaeocystis sp.* and some crysophytes (Jeffrey 1997). The pigment Chl c3 is mainly associated to haptophytes and some dinoflagellates (Jeffrey 1997). Hence a decrease of these pigments should reflect a decrease of these phytoplanktonic groups.

The ratios of 19'-hexanoyloxyfucoxanthin to fucoxanthin (hexa:fuco), fucoxanthin to chlorophyll a (fuco:Chl *a*) and chlorophyll c3 to fucoxanthin (Chl c3:fuco) were calculated to visualize changes in species composition (Figure 3.10).

No changes in pigment ratios in response to the pCO_2 treatment were observed. However, at the beginning of the experiments A, C, D and F hexa:fuco was < 0.5, while a hexa:fuco ratio > 0.5 was found in experiments B and E (Figure 3.10A). Despite the initial differences at the beginning of the experiments, hexa:fuco ratios declined to approximately zero in all experiments. Although low hexa:fuco have often been interpreted as an indication for the prevalence of diatoms, their susceptibility to Fe and light makes them unsuitable to distinguish between changes in species composition and culturing effects in our experiments.

The ratio fuco:Chl *a* was unchanged during most of the experiment and increased at the end (Figure 3.10B). Because addition of Fe, decreasing irradiance and changes of the species composition towards a diatom-dominated phytoplankton community affect this ratio in the same manner, it is also not suitable for drawing conclusions about the species composition in our incubations. However, as fucoxanthin is known to function as a light harvesting pigment (van Leeuwe *et al.* 1998), we argue that the observed increase at the end of the experiments represents the first sign of light limitation. [Chl *a*] increased to approximately 25 $\mu g\ l^{-1}$ at the end of the experiments, which has been shown to be sufficient for self-shading of oceanic phytoplankton (Morin *et al.* 1999).

Chl c3 relative to fucoxanthin data (Figure 3.10C) indicates that prymnesiophytes were only present in small numbers in the experiments. The pigment Chl c3 was found in relatively small amounts at the beginning of the experiments. Despite the fact that the synthesis of Chl c3 is stimulated upon addition of Fe (Di Tullio *et al.* 2007), the pigment concentration decreased even further indicating that also the haptophytes in the incubations decreased. Still, the abrupt decrease of Chl c3:fuco immediately after the start of the experiments indicates that culturing conditions also influenced this pigment ratio.

Based on pigment composition no differences between the treatments were found indicating that pCO_2 did not influence species composition. We conclude that other factors such as Fe addition and light affected pigment composition, but

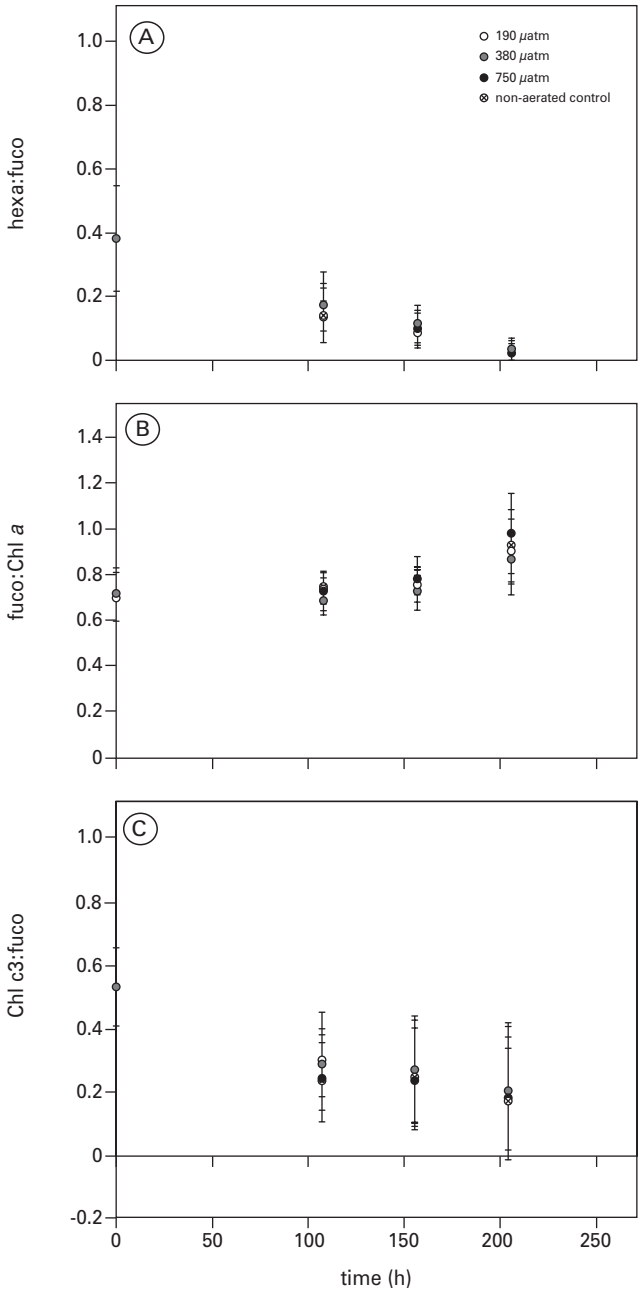


Figure 3.10. Pigment ratios (hexa:fuco, fuco:Chl *a*, Chl c3:fuco) of several marker pigments for the experiments A - F.

that it is not possible to disentangle to which extent. Data on acquisition of C, presented in Chapter 4, indicates that Fe indeed had a larger impact on species composition than pCO_2.

Based on pigment ratios, we suggest that prymnesiophytes played a modest role in the experiments and diatoms progressively dominated the incubations. This is also supported by data on nutrient depletion (Table 3.4). If species composition during the experiments shifts towards a different relative contribution of diatoms, this has potential impact on nutrient ratios. We therefore examined ΔN:ΔP, ΔSi:ΔN and ΔSi:ΔP depletion ratios in time. Uptake ratios were typical for diatom-dominated communities with the exception of experiment F. No major shifts in ratios were observed over time (data not shown), indicating that diatoms were the major phytoplankton taxon throughout the experiments.

Diatoms play a major role in global carbon fixation in all regions of the world's ocean (Sarthou *et al.* 2005), and tend to dominate the phytoplankton community in numbers and in production in the Southern Ocean, accounting for as much as 75 % of the annual primary production (Nelson *et al.* 1995). In our bottle incubations relatively high and stable light conditions (compared to the situation in the field) prevailed, which might have favoured diatom growth. In a field study Arrigo *et al.* (1999) observed that diatoms became dominant under stratified conditions, while *Phaeocystis* dominated in mixed waters. The authors argued that this might be a consequence of diatoms being able to grow in relatively high light conditions, whereas *Phaeocystis* prefers lower light levels.

Table 3.4. Nutrient depletion ratios (ΔSi:ΔN, ΔSi:ΔP and ΔN:ΔP) during experiment A - F. All data from the different CO_2 manipulations (190, 380, 750 µatm pCO_2 and control) were pooled per experiment and fitted with linear regression. The slopes represent nutrient depletion ratios. R^2 indicates goodness of fit.

		ΔSi:ΔN	ΔSi:ΔP	ΔN:ΔP
Experiment A	Slope	1.24 ± 0.02	15.60 ± 0.79	12.73 ± 0.63
	R^2	0.98	0.87	0.87
Experiment B	Slope	1.34 ± 0.03	17.98 ± 0.40	20.65 ± 0.46
	R^2	0.97	0.97	0.97
Experiment C	Slope	1.30 ± 0.02	19.04 ± 0.74	14.43 ± 0.51
	R^2	0.97	0.92	0.93
Experiment D	Slope	1.17 ± 0.03	15.56 ± 0.34	13.16 ± 0.24
	R^2	0.95	0.96	0.97
Experiment E	Slope	0.90 ± 0.02	13.19 ± 0.32	14.55 ± 0.34
	R^2	0.93	0.93	0.94
Experiment F	Slope	0.67 ± 0.02	9.93 ± 0.32	14.78 ± 0.47
	R^2	0.92	0.92	0.93

An alternative explanation might be that the silicified diatoms were able to deal better with the turbulence caused by aeration than the prymnesiophytes. In this case, the hexa:fuco ratios should differ between aerated and non-aerated control bottles. No differences between aerated and non-aerated control bottle were found, indicating that aeration did not influence species composition in the incubations.

In pCO_2 manipulation experiments performed with natural phytoplankton assemblage from the Ross Sea, Tortell *et al.* (2008) observed a change of species composition within the same taxon. Under conditions of low [CO_2] pennate diatoms such as *Pseudonitzschia* dominated, whereas under high [CO_2] conditions large, chain-forming *Chaetoceros* species were most abundant. Changes in the species composition on the genus level were not assessed in this study.

Generally, changes in pigment ratios might also be a result of changes in cell size as larger cells also contain larger amounts of pigment. A comparison of growth rates derived from flow cytometry and *in vivo* fluorescence data indicated that in this study the phytoplankton community shifted to larger cells.

Effect of elevated [CO_2] in the incubations

Several parameters (nutrient uptake, growth rates of cells < 20 µm and POC) indicated that phytoplankton in the incubations grew faster under high pCO_2. In opposition to this finding no significant differences between the pCO_2 treatments were found in Chl *a* concentration and chlorophyll fluorescence yield after dark adaptation (F_0).

A significant linear increase in the uptake of N, P and Si from low to high pCO_2 and significant differences between the treatments were observed in the uptake rates of Si and P. Although not significant, the same trend was observed for N uptake. The increase in nutrient uptake with increasing pCO_2 suggests that phytoplankton

Table 3.5. Effects of environmental factors and change in species composition on several pigment ratios. An increase in ratios is represented by ∧, a decrease by ∨. *1: both pigments increase, *2: no data on light effect on Chl c3.

Environmental factor or change in species composition	Increasing Fe	Decreasing irradiance	Increase of diatoms/ decrease of prymnesiophytes
Pigment ratio			
hexa:fuco	∨	∨	∨
hexa:Chl *a*	∨	*1	∨
fuco:Chl *a*	∧	∧	∧
Chl c3:Chl *a*	∨	*2	∨
Chl c3:fuco	∧	*2	∨
hexa:Chl c3	∨	∨	*2

grew faster under high pCO_2. This is supported by growth rates derived from cell numbers determined by flow cytometry over the course of the experiments (Table 3.2B). These growth rates differed significantly between treatments, following a linear trend from low to high pCO_2 (190 < 380 < 750 µatm). With an average of 0.3 d^{-1} they compared well with growth rates calculated for the same incubations from short-term $^{14}CO_2/H^{14}CO_3^-$ disequilibrium experiments (Chapter 4). They are also in good agreement with other bottle incubations with Southern Ocean phytoplankton assemblages (Veldhuis & Timmermans 2007).

Phytoplankton also accumulated significantly more POC when incubated at high pCO_2. In general, the higher accumulation of POC in the high pCO_2 treatments might be a result of higher POC accumulation within the cells and/or higher biomass accumulation in the incubations. Higher POC accumulation within the cells should be reflected in a higher POC content per cell, but assessment of POC:cell for cells < 20 µm provided no evidence that cells stored more carbon when grown under high pCO_2. A higher accumulation of POC:cell for cells > 20 µm cannot be excluded.

Opposing with this finding, no significant differences between the pCO_2 treatments were observed in terms of Chl *a* and, accordingly, in the chlorophyll fluorescence yield after dark adaptation (F_0). At the beginning of the experiments the concentration of Chl *a* varied between 0.10 - 0.69 µg Chl *a* l^{-1} and, hence, were typical for Southern Ocean HNLC conditions. The highest concentration was observed in experiment A at the Polar Front. Ultimately the concentration of Chl *a* reached approximately 25 µg l^{-1} in the incubations. Alderkamp *et al.* (2010) reported similar Chl *a* concentrations for surface waters during the same cruise: 0.2 - 0.8 µg Chl *a* l^{-1} along the Greenwich Meridian and 0.2 - 0.6 µg Chl *a* l^{-1} in the Weddell sea.

No significant differences between the pCO_2 treatments were observed. We speculate that Chl *a* was mainly influenced by the addition of iron. This view is supported by carbon acquisition data from the same incubations presented in Neven *et al.* (2011) and the fact that POC:Chl *a* decreased by approximately 50 % during the experiments despite the relative high-light conditions (Figure 3.8C).

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